

Adapting the methylene blue sulfide detection method for use in a microfluidic in situ monitoring device.

Shane W. Monares

James D. Ingle Jr. (Mentor)

Department of Chemistry, OSU, Corvallis, OR 97331

Goals

- Study and assess the stability of sulfide, S(-II), solutions
- Explore protection techniques for sulfide solutions
- Prepare and test a single, multi-component reagent solution capable of performing the methylene blue reaction
- Adapt the mixed reagent to work in an in situ microfluidic device
- Use the modified method in the microfluidic device to measure sulfide in the laboratory with real environmental samples and in the field

Chemistry

- Simply, the organic reagent N,N-dimethyl-p-phenylenediamine (DMPD) is oxidized by iron (III) which reacts with hydrogen sulfide (H₂S) to form methylene blue (MB) as seen in Figure 1.¹

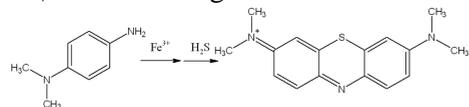


Figure 1. Basic mechanism of the MB reaction

- MB strongly absorbs visible light in the 600-800 nm region of the electromagnetic spectrum. In the MB reaction the amount of sulfide in the sample is directly related to the amount of MB formed.

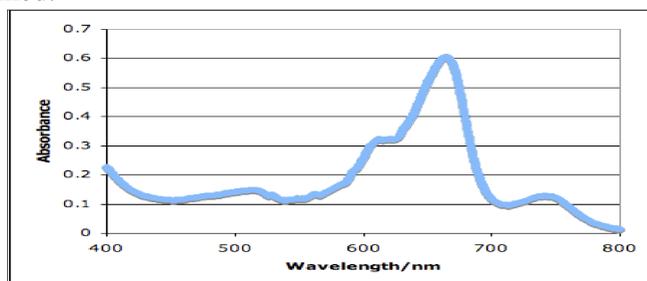


Figure 2. Methylene blue spectrum formed from the reaction of 20 μ M S²⁻ and the mixed reagent. The pH of the MB is ~1. Measurements are taken at the 664 nm maximum. The maximum at 742 nm is the protonated MB species and becomes dominant at much lower pH.

Acknowledgements

Feel free to E-mail me at monares@onid.orst.edu should you have any questions.
Funding for this research provided by the Subsurface Biosphere Initiative.
Funding for the Ocean Optics spectrometer provided by SG-05-085 Camille and Henry Dreyfus Foundation Special Grant in Chemical Science.



Instrumentation

- For bench-top laboratory purposes the scheme seen in Figure 3 was used throughout. Figure 4 is microfluidic device for which this new method is being developed.

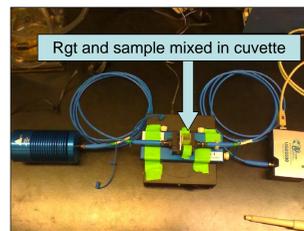


Fig. 3. From left to right: Halogen lamp, fiber optic cable, solution mixer/cuvette holder, fiber optic cable, Ocean Optics spectrometer, USB cable to PC.

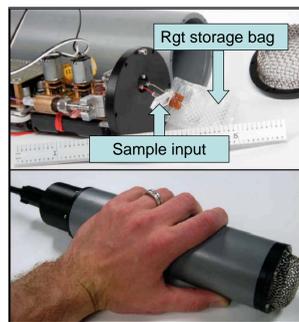
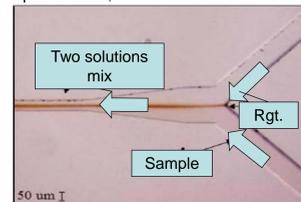


Figure 4. The micro-total analysis device developed by Corey Koch. The device is water submersible and able to take measurements in situ.³

Fig. 5. Microfluidic Y-mixer. One channel is used for the sample, one for the reagent. Theoretically, MB will form once the two are mixed. Image by Corey Koch.³

Reagent Preparation

- A single mixture of 2 reagents in an acidic medium was used throughout the majority of the studies (Table 1). The DMPD/Fe(III) mixed reagent (denoted standard reagent) was prepared using directions given by Cline.² A mixed reagent is necessary due to pumping and storage limitations unique to the in situ device.

Table 1. Reagent and standard concentrations adapted from Cline's method (1969).

	Mixed Rgt	Conc. (mM)	Cuvette Conc. (mM)	Cuvette moles (μ mol)
0.15 mL Rgt	Fe(III)	22.0	1.63	3.26
	DMPD	17.9	1.33	2.65
	HCl	3.88 M	287	574
1.85 mL S(-II)	Std	Conc. (mM)	Cuvette Conc. (mM)	Cuvette moles (μ mol)
	S(-II)	20 μ M	19 μ M	0.037
	KOH	10	9.26	18.5

Sulfide Stability

- For safety and measurement purposes a sulfide anion stabilizer was needed. A simple addition of concentrated base to the standards had the lowest effect on MB signal strength and eliminated some loss of sulfide due to oxidation. Ascorbic acid was not used because of its effect on the signal.

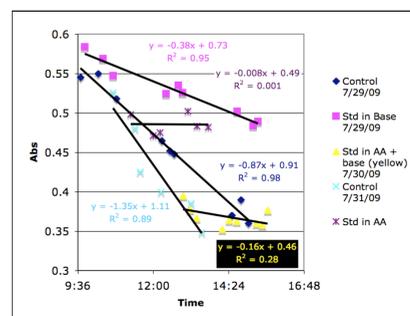


Figure 6. Methylene blue signals measured at 664 nm. Stability of 20 μ M sulfide standards using no protection (light and dark blue), as a pH 12 base (pink squares), and ascorbic acid in base (yellow and purple asterisks). The signal and reproducibility with the addition of ascorbic acid in the basic solution are lowered.

Adaptation of Method

- A mixed reagent was prepared to achieve a final pH of ~0.8. Cline's method uses a 12:1 sample to reagent dilution. Work was done to create a reagent that would function with a 1:1 sample to reagent dilution. Dilution of the standard mixed reagent raised the pH and the solution prepared was pink (Figure 7).



Figure 7. This pink form was found to be unreactive when sulfide was introduced.



Figure 8. From left to right: 50% (dilution of std reagent with water), std reagent, 200% (double conc. of all components in std reagent). Upper - the solutions as they were initially prepared. Lower - the transformed 50% reagent in an unreactive form.

- Standard reagent diluted with water to 70% or less of the concentrations of the std reagent equilibrated to the less reactive pink/purple forms in a matter of hours (see Fig. 8). Adding concentrated acid to the mixed reagent minimizes the conversion of DMPD to a non-reactive form. Figure 9 shows the spectra of the MB when 20 μ M S(-II) standards were reacted with each reagent species.

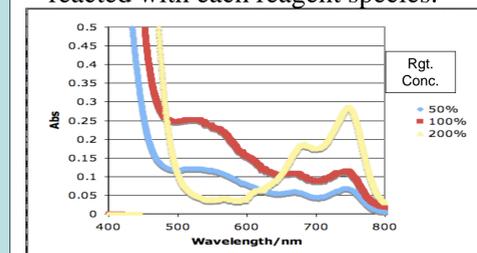


Figure 9. MB spectra from 50% (dil. W/ 3.88 M HCl), 100%, and 200% reagent concentrations reacted with 20 μ M S²⁻ samples in a 1:1 dilution. The tailing at 400-500 nm is not seen in the typical MB spectrum. For the most concentrated reagent, the 742 nm peak is dominating, but the signal is strong.

Conclusions

- Sulfide standards that are unprotected are readily oxidized by air and can not be used for measurement purposes over the course of a day.
- Sulfide standards protected with 0.01 KOH had a loss in signal of just 16.2% compared to 33.9% for unprotected stds.
- Dilution of Cline's mixed reagent results in a degradation of the reactive species.
- The mixed reagent is stable only at when the reagent mixture acid concentration is ~3 M or greater.

Future Studies

- Studying long term stability of highly acidic reagents.
- Explore the possibilities of using the 742 nm peak in the MB spectrum instead of the 664 nm peak normally measured.
- Determine if a highly acidic reagent can be stored in the reagent bag if the microfluidic device safely.
- Measure simulated and real environmental samples for S(-II) content.

References

1. Kuban, V., Dasgupta, P. K., Marx, J. *Anal. Chem.* **1992**, 64, 36-43.
2. Cline, J.D. *Limnology. and Oceanography.* **1969**, 14, 454-458.
3. Koch, Corey. Micro Total Analysis System for *In-situ* and Autonomous Spectrophotometric Monitoring of Iron in Groundwater. Ph. D. Dissertation, OSU, Corvallis, OR. 2009.